

**Report to South Dakota Game Fish and Parks**

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**Common Nighthawks (*Chordeiles minor*) in agricultural landscapes: Thermal microclimates, nesting productivity and population genetic structure**

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## Abstract

Grasslands and riparian forests in southeastern South Dakota have been greatly reduced since historical times, and this decline has recently intensified due to conversion to agricultural row-crop production and dam control of the Missouri River's flood pulse. Common Nighthawks' (*Chordeiles minor*) nesting habitat includes grasslands, open woodlands and urban rooftops, but nesting sites in our study area are limited to rooftops due to this land use change. The study had two related objectives. First, we located and monitored nighthawk nests at urban rooftop study sites to document relationships among nest thermal microclimates and nestling condition (measured by plasma corticosterone levels). Second, we examined genetic relatedness among chicks, within clutches and from different rooftops and towns (i.e., Elk Point, North Sioux City, Vermillion, and Yankton) in the study area to determine population genetic structure and gene flow of nighthawks in the study area. These data will help predict the impact of agriculturally dominated landscapes on chick survival and genetic diversity and contribute important information on how land use practices impact nesting ecology and conservation for this at-risk species. Rooftop habitats can vary in magnitude of elevated temperatures over neighboring vegetated land cover types, which might have an effect on chick condition. Using multiple regression we analyzed regional climate data during the local nighthawk breeding season (May 15-August 15) from three surrounding weather stations and found increasing mean humidity (%) and decreasing mean wind (KPH) and cloud cover (%) 1948-2016 in at least two weather stations' data (all  $P < 0.05$ ). Despite the changing regional climate, we found no notable significant relationship between the chick condition response variables (i.e. baseline, stressed and magnitude of stressed corticosterone [CORT] response), and predictor variables chick condition (i.e. mass and wing length), nest microclimate (i.e. temperature and wind), regional climate (i.e. temperature, dew point, humidity

and wind for the past day, week and 30 days) for 24 rooftop chicks from 17 nests 2015-2016, with the exceptions of  $\log\text{CORT}_B$  size significantly increasing with increasing anemometer ambient temperature ( $T_a$  °C) ( $P<0.001$ ,  $F_{1,13}=22.81$ , Adj.  $r^2=0.609$ ,  $\text{Coef}=0.075$ ) and with increasing ovoid operative temperature ( $T_e$  °C) ( $P<0.05$ ,  $F_{1,12}=5.318$ , Adj.  $r^2=0.249$ ,  $\text{Coef}=0.0450$ ). This suggests nighthawks, much like nightjars in general, are largely adapted to current climatic conditions as a result of recent regional changes in humidity, wind and cloud cover. Using DNA extracted from 25mg tissue, blood, feather and fecal samples from 38 eggs, chicks and adults found in the same urban population, we tested relatedness between subpopulations of four towns. We amplified the samples via PCR using mitochondrial DNA primers and sequenced the results using FinchTV 1.4.0 and aligned the data using ClustalW in BioEdit 7.2.5. We analyzed the genetic relatedness between subpopulations using a Chi-square test for differences in haplotype frequencies and found relatedness (pairwise  $F_{ST}\leq 0.11$ ) between three subpopulations (i.e. Yankton, Vermillion and North Sioux City) found along the Missouri River and distinctive haplotypes (pairwise  $F_{ST}=0.04, 0.15, 0.19$ ) in the fourth subpopulation (i.e. Elk Point). These preliminary results suggest low gene flow between Elk Point and most of the other towns due to segregated nesting habitats as a result of agricultural intensification in the terrestrial land cover types between Elk Point and the other towns. Moreover, these preliminary results suggest gene flow is enabled by the relatively undisturbed riverine land cover types that connect the genetically similar Yankton, Vermillion, and North Sioux City subpopulations. This will contribute important information on susceptibility to climate change and genetic isolation of an at-risk species in the Northern Prairie region.

## Project Timetable

Date	Milestones
May-Aug 2014-2016	Nest searches and monitoring, collection of blood samples from chicks and salvage of any dead eggs and chicks
Aug-Dec 2016	Conduct corticosterone and genetic assays
Jan-April 2017	Data analysis and compilation of report findings
28 April 2017	Final report submitted

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**Key words:** Corticosterone, Common Nighthawk, nestling condition, microclimate, regional climate, stress response, gene flow, land use change

## Introduction

Since 1980, aerial insectivorous birds have declined in numbers throughout North America. As a guild, aerial insectivores have experienced a greater decline than songbirds (Sauer et al. 2007). The decline of aerial insectivores potentially has large-scale ecological impacts, since insectivores provide important ecosystem services, such as agricultural and residential pest control.

The taxonomic order Caprimulgiformes is a large taxon that includes aerial insectivores, but is one of the least studied avian clades worldwide due to their nocturnal and crepuscular

nature (Cleere 1998). Common Nighthawks are one of the more studied species of Caprimulgidae, yet much of the information relative to their natural history is anecdotal (Brigham et al. 2011).

Common Nighthawks have a wide breeding distribution in North America, yet are subject to local population declines. North American Breeding Bird Survey data from 1966-2013 indicate a declining population trend for Common Nighthawks in North America, with a 1.9 % decline annually for North America and a 1.2 % decline annually for South Dakota (Sauer et al. 2014). The annual South Dakota trend from 1980-2006 was -4.3%, showing an accelerated decline relative to 1966-1979 (-0.9% annually, Sauer et al. 2007). This accelerating decline in population for South Dakota is coincident with a period of climate change in the Northern Prairie region (i.e. associated with earlier springs that might affect insect phenology) (Swanson and Palmer 2009). Populations throughout most of the Western Corn Belt states (i.e. Minnesota, Iowa, North Dakota, and South Dakota) share the same downward trend in populations (Sauer et al. 2014).

Due to changing land use practices, natural nighthawk nesting sites (i.e. open woodlands and grasslands) in the Northern Prairie region are in decline (Tallman et al. 2002). The study area, southeastern South Dakota, is currently dominated by row-crop agriculture, but was historically covered by grasslands (Spess Jackson et al. 1996, Tallman et al. 2002) and is within the area showing the greatest recent loss of grassland in the Western Corn Belt region (Wright and Wimberly 2013). This conversion of grassland to row-crop agriculture has accelerated recently, with the higher prices for corn and soybeans 2006-2013 (Wright and Wimberly 2013). Such conversion and loss of natural habitat is likely driving greater use of urban flat, gravel rooftop habitat by nighthawks and likely contributes to population declines for this species.

Common Nighthawks sometimes use flat, graveled, urban rooftops for nesting.

Environmental disturbance of natural nesting sites in agricultural landscapes and abundant insect prey surrounding urban light fixtures influence nest choice for Common Nighthawks (Brigham 1989). Natural habitat choice is associated with high grassland cover (Ng 2009), suggesting that early 20th Century and ongoing conversion of South Dakota's tall-grass prairie to agriculture (Tallman et al. 2002) might be causing Common Nighthawks to be displaced to urban settings.

Natural nest sites typically occur in grasslands or open woodlands with minimal disturbance (e.g., little agricultural disturbance, limited pesticide use) (Brigham 1989, Wedgewood 1991), which are preferred over closed forests to allow for more air movement and facilitate heat loss (Fisher et al. 2004). Urban nests are typically located on flat, graveled rooftops, but these sites are not used uniformly throughout their range (Brigham 1989, Brigham et al. 2011). Preferences for habitat can be influenced by long-term pressures including habitat disturbance and nest microclimate changes as well as by proximate pressures such as food availability (Chalfoun and Schmidt 2012). The use of gravel roofs suggests that abundance of prey in urban settings (e.g., around light fixtures) might outweigh costs associated with urban nest sites (Brigham 1989), including increased predation risk from urban generalist predators such as raccoons, corvids, and domesticated cats (Marzilli 1989, Wedgewood 1991).

Because Common Nighthawks are opportunistic feeders (Caccamise 1974), their diet is based upon prey availability (Todd et al. 1998) and is expected to differ between grassland, open woodland and urban habitat types. Because insect availability may be limited by weather, insectivores are less active during extreme weather events (i.e., cold, rain). This can decrease foraging opportunities during the critical breeding period. Furthermore, changes in Missouri River flow patterns following the completion of dams in the 1950s have resulted in declines of

macroinvertebrates in Missouri River riparian habitats in South Dakota (Hay et al. 2008). Thus, the combination of river regulation and agricultural land conversion in South Dakota areas bordering the Missouri River, including the study sites, are likely to have a reduced abundance of insects. These changes might contribute to nighthawks seeking alternative nesting sites, such as urban rooftops. However, for our study region, this stretch of the Missouri River has been set aside as a National Recreation River, which might aid nighthawks in migration, foraging and gene flow between towns.

Nevertheless, because of the potential stressors, the Common Nighthawk may serve as an effective indicator of habitat health. Habitats can affect adult condition by influencing reproductive success, body condition, and the immune system (Wingfield et al. 1992, Wingfield et al. 1997). Perturbation of habitat can produce elevated baseline levels of the stress hormone corticosterone ([CORT], the primary stress hormone in birds) and lead to a delay or halt of the breeding season (Wingfield et al. 1994, Schoech et al. 2008). Elevated baseline corticosterone can also activate immunosuppressants in egg-laying females, which can be incorporated into the yolk, thereby impacting condition of the nestlings (Love et al. 2005). By comparing the birds' stress hormone response among urban rooftop sites, we can ascertain mechanisms by which urban habitats might affect reproductive success and thus, fitness.

Our research evaluated nest conditions (i.e. microclimate) and plasma corticosterone of chicks at rooftop sites and correlated these traits with a variety of climatic variables. While other Caprimulgids have shown a tolerance for elevated temperatures (Cowles and Dawson 1951, Howell 1959, Bartholomew et al. 1962, Lasiewski and Dawson 1964, O'Connor et al. 2016), and nighthawks have moderate vulnerability to climate changes in temperature and humidity in other regions (i.e. Upper Midwest Great Lakes, Culp et al. 2017), nest microclimate is important to

minimize heat loss in cold weather and maximize heat loss during higher temperatures (Kortner and Geiser 1999), so similar effects of thermal microclimates at nest sites might be expected for Common Nighthawks.

Because Common Nighthawks require specific nest microclimates that allow for more air movement to facilitate heat loss (Fisher et al. 2004), a preference for habitat with high temperatures has the potential to become an ecological trap (Fletcher et al. 2012) if climate change produces even higher temperatures. Great Plains mean temperatures are expected to increase by 3.6°C – 6.1°C over the next 100 years (Ojima and Lockett 2002). As a result of climate change, ranges for Great Plains bird species are expected to be reduced by 35% (Peterson 2003). At the nearest weather station to some of our field sites, Sioux City, Iowa, mean summer temperatures is expected to increase by 6.3°C by the year 2100 and the mean summer dew point there is expected to increase by 1.1°C (Kenward et al. 2014). Projected climate changes worldwide require rates of evolution greater than 10,000 faster than has been observed for most organisms (Quintero and Wiens 2013). Seasonal differences have been shown in the adrenocortical response in breeding birds (Wingfield et al. 1992) by suppressing the stress response in some species during the hottest days. Yet, short term temperature changes have been associated with increased plasma corticosterone concentrations in non-adapted captive-reared turkeys (El-Halawani et al. 1973). As a result, nighthawks might be adapted to heat fluctuations within the normal range, but not within projected climate change scenarios.

Urban habitats often function as “heat islands,” with modified regional climate due to reduced vegetation cover, impervious surfaces, and a high density of buildings, which lower evaporative cooling, store heat, and warm the surface air (Bonan 2002). Urban areas in the Midwest and Great Plains temperatures are 2.4°C greater than neighboring rural areas (Kenward



et al. 2014). Exposed urban rooftop nest sites thus have the potential to produce thermally unfavorable conditions for nesting nighthawks, but no recent studies have examined nest microclimates or nestling condition for nighthawks at urban rooftop nesting sites. Moreover, gravel rooftop surfaces might promote lower temperatures than other rooftop surface types. For example, Marzilli (1989) found that a simulated rubberized rooftop surface was cooled from 56.3 °C to 41.6 °C with the addition of gravel.

If Common Nighthawks are displaced to urban sites and climate change is likely to produce microclimate temperatures too high for successful nesting in urban areas, this could negatively impact Common Nighthawk populations, and leave them with few alternatives due to the reduction of natural nest site availability because of anthropogenic habitat disturbance. Bird body temperatures typically range from 39°C to 42 °C, but body temperatures reach lethal levels at approximately 46 °C (McNab 2002). If nest microclimates are hot enough that birds spend substantial energy on evaporative cooling or face conditions where such cooling is insufficient to prevent rising body temperatures, this could affect a bird's ability to successfully incubate eggs and hatch chicks. For example, when week-old nighthawks were exposed to direct sunlight at a 42 °C, chick body temperatures rose to 44°C and chicks showed "great distress" (Lohnes 2010). While nightjars are capable of greater heat tolerance than passerines (Whitfield et al. 2015), roof parapet shade and higher wind speeds (Cooper 1999) at rooftop nest sites than at sites closer to the ground might help mitigate high temperatures, and urban rooftop sites could present nesting refugia for nighthawks displaced from natural habitats by anthropogenic disturbance. Or, conversely, reduced cloud cover can exacerbate radiance (Wielicki et al. 1995) and thus, increase temperatures at exposed rooftop sites.

Determination of thermal microclimates (i.e., operative temperatures [ $T_e$ ] = an integrative measure of the actual thermal environment encountered by an animal, including radiative and convective heat exchange; Walsberg 1986) and their influence on nesting success for urban rooftop nests will provide valuable information on the effects of natural habitat loss on Common Nighthawk nesting ecology. In addition, correlations among body condition indices and operative temperatures will help inform predictions for how landscape and climate change might impact nighthawk population ecology. Such data will greatly benefit conservation of this declining species.

The study's first objective was to determine the association between chick condition (i.e. morphometrics, corticosterone), regional climate, rooftop conditions and thermal nest microclimate for urban rooftop nest sites. Panting and gular fluttering at high temperatures increase metabolic rates but promote evaporative cooling in nighthawks (Lohnes 2010). High temperatures at nest sites coupled with high humidity result in challenges to nestlings, both from thermoregulatory (evaporative cooling) and water balance standpoints (Gerson et al. 20014, Lohnes 2010). We hypothesized that chicks at nests with greater operative temperatures will have higher baseline stress hormone (CORT) levels.

Nighthawk occupancy of urban rooftop nesting sites is affected by rooftop characteristics (Viel 2014). Because of higher wind speeds at rooftop rather than at ground sites, the convective advantages of rooftop sites might help mitigate the higher temperatures experienced at rooftops compared to ground nest sites (Fisher et al. 2004, Fletcher et al. 2012). However, the characteristics of the parapet surrounding rooftop nest sites may also affect microclimates and nest success, but not in a straightforward manner, as a trade-off between thermal microclimate and falling danger likely exists. High parapets on rooftops will likely reduce wind movement

resulting in warmer thermal microclimates at the nest. Thus, we predicted that sites with higher rooftop heights and lower rooftop parapet heights will have more suitable microclimates with lower operative temperatures ( $T_e$ ). As a consequence these nesting sites are predicted to produce lower baseline stress hormone (CORT) levels than those with lower rooftop heights and higher parapet heights.

Baseline CORT levels in nestling birds are sometimes positively correlated with exposure to high ambient temperatures within the nest (Lohnes 2010). Such exposure can lead to reduced survival and fitness by downregulating the immune system or mobilizing energy stores (Lohnes 2010). We hypothesized that baseline CORT levels of chicks will be positively associated with the maximum operative temperature encountered at the nest and to the duration of exposure to operative temperatures in excess of the lethal body temperature threshold of approximately 46°C (McNab 2002).

Moreover, a population genetics study of this species to examine the relationship between gene flow and distance between geographically isolated nesting sites has never been conducted. Using mtDNA sequence data to estimate the level of genetic variability, number of maternal lineages, and effective population sizes, we will be able to assess the potential that our sampled populations are suffering from inbreeding depression. Inbreeding depression occurs in small, isolated populations and results in poor population performance, including depressed fecundity and low population growth rates (Allendorf and Luikart 2007, Frankham et al. 2010). These results, in conjunction with demographic data, will inform predictions for how land use change might impact nighthawk population ecology and provide valuable insight into management plans for nighthawks in the Northern Prairie region, which should greatly benefit conservation for this declining species that provides important ecosystem services.

The study's second objective was to determine the population genetic structure of nighthawk chicks, within clutches and from different rooftops and towns (i.e., Elk Point, North Sioux City, Vermillion, and Yankton) in the study area, to determine the relationship between gene flow and distance for nighthawks in the study area. This objective was in collaboration with Dr. Hugh B. Britten, Department of Biology, University of South Dakota, who has expertise in animal population genetic analyses.

## **Material and methods**

### **Nest searches**

We surveyed Google Earth for gravel rooftops near sites where nighthawks were present during point counts (Common Nighthawks [*Chordeiles minor*] in the Western Corn Belt: Habitat Associations and Population Effects of Conversion of Grassland and Rooftop Nesting Habitats; manuscript in prep) in North Sioux City, Elk Point, Vermillion and Yankton, South Dakota, and then searched identified rooftops for nesting birds and nest sites (Figure 1). Systematic searches of rooftops for nests involved laying out a grid network with 1 m x 1 m squares on graveled areas of the rooftop and walking all gridlines until adult birds flushed. When adults flushed, we carefully searched the area where the adult flushed for eggs or chicks.

### **Corticosterone collection and measurement**

For CORT measurements, we removed chicks from the nest by hand at approximately 14 days after hatching and collected blood samples (<100 microliters) by pricking the brachial vein with a 26-gauge needle and collecting blood in a heparinized capillary tube. Following collection of the blood sample, we applied pressure to the bleeding site with a cotton ball until the bleeding stopped. Birds were subjected to two blood draws (<100 microliters each), one from each wing,

for measurement of the stress response (i.e. increase in corticosterone following handling stress). Following the first blood sample, individual birds were placed in cloth bags in a shaded location for 30 minutes after the first blood draw, upon which the second blood draw was collected. These sampling methods (Wingfield et al. 1994) are standard for drawing small volumes of blood from birds and are approved methods in the Ornithological Council's (2010, 3rd edition) *Guidelines to the Use of Wild Birds in Research*. Blood samples were stored on ice in microcentrifuge tubes while in the field.

Upon return to the laboratory, we centrifuged blood samples for 10 min at 3000 X g at 4 °C, drew off the plasma, and stored plasma (and red blood cells for the genetics study) frozen at -80 °C until later analyses via a commercially available spectrophotometric kit (ELISA kits ADI-900-097), as previously conducted in our laboratory (Liu and Swanson 2014). Following blood collection, we banded chicks with a standard USFWS aluminum leg band, performed morphometric measurements, and released them back to their last nest site. For morphometrics, we measured unflattened wing chord length with calipers to the nearest 0.1 mm and measured mass to the nearest 0.5 g with a Pesola spring balance. We sampled 24 nestlings from 17 nests 2015-2016.

### **Nest microclimate**

Nest microclimate data was collected using two methods. First, iButton data loggers (DS1921G-F5# Thermochron) were placed in the nest scrape next to the chicks and moved every week to where the eggs and chicks have relocated and recorded ambient temperature ( $T_a$ ). We programmed the iButtons to record nest temperature (°C) every 10 minutes from incubation to fledging (Ardia et al. 2006). Second, we deployed operative temperature ( $T_e$ ) thermometers within 1-2 meters from the eggs or chicks (moved every week to where the eggs and chicks have

relocated) at sites with similar conditions to the nest site (e.g., shade prevalence and amount of gravel substrate). This distance from the nest site is far enough away to avoid disturbing the birds (our unpublished work). Operative temperature ( $T_e$ ) thermometers were designed from copper ovoids using 10 cm x 12 cm copper toilet floats (approximately the same volume as adult nighthawks) with the outside surface painted flat gray. We cut a 2.5-cm hole in the copper ovoid to attach a 2-m beaded thermocouple sensor probe (with Type-T mini connector) and placed inside the copper ovoid connected to a Model UX120006M 4-channel analog input HOBO data logger (Hobo Instruments, Contoocook, NH). We recorded operative temperature once per minute from egg discovery date to chick fledging. Third, during weekly nest visits, we placed a CIH20DL Data Logging Hot Wire Anemometer with CFM/CMM and 8 to 1 infrared thermometer (General Tools & Instruments, Secaucus, NJ) adjacent to the chicks or eggs to record maximum wind speed (KPH) and maximum temperature to allow estimation of convective heat loss.

### **Regional climate**

We collected regional climate data, i.e. hourly temperature ( $^{\circ}\text{C}$ ), relative humidity (%), dew point( $^{\circ}\text{C}$ ), visibility (km), maximum wind gust (KPH), precipitation (cm), cloud cover (%), and wind speed (KPH), during the Common Nighthawk breeding season May 15-August 15 (our unpublished work) 1948-2016 from the three nearest weather stations in Yankton and Sioux Falls, South Dakota, and Sioux City, Iowa (NOAA 1948-2016) (Figure 1).

### **Roof characteristics**

We calculated the roof height (m) by measuring distance to the building base and roof top using a Bushnell Yardage Pro Sport 450 distance meter. We measured the height of the roof parapet and a sample of three roof gravel substrate pieces using calipers to the nearest 0.1 mm.

## Corticosterone Study Statistics

We performed a multiple regression analysis of annual mean of maximum, minimum and mean of daily regional climate recordings 1948-2016 using R 2.13.2 with the car package (Fox and Weisberg 2011). We compared rooftop characteristics (as predictor variables) and microclimate dependent variables at the time of blood collection using multiple regression in R 3.3.2 and condition in chicks among and within nests (i.e. chicks were assigned a 0 or 1, based upon order of blood collection) using multiple regression in R 3.3.2 with three response variables: 1) log-transformed baseline CORT ( $\log\text{CORT}_B$ ), 2) log-transformed CORT response to restraint stress ( $\log\text{CORT}_{30}$ ) and 3) the log-transformed magnitude of CORT response to restraint stress relative to the baseline CORT ( $\log\text{CORT}_{30}-\log\text{CORT}_B$ ). We evaluated the association between the response variable and the predictor variables using five models: temporal (i.e. Year, Julian Date with Year, Julian Date, Decimal Time [hours since midnight]), rooftop conditions (i.e. roof parapet height [cm] above roof surface, roof height [m] above ground, and mean diameter of three random pieces of gravel [cm] surrounding the nest site), chick condition at blood collection (i.e. mass [g], wing length [cm]), nest microclimate (i.e. maximum anemometer temperature [ $^{\circ}\text{C}$ ] and wind speed [KPH] at blood collection, iButton temperature [ $^{\circ}\text{C}$ ] at blood collection, ovoid operative temperature [ $T_e$ ] [ $^{\circ}\text{C}$ ] at blood collection), and regional climate (i.e. maximum temperature [ $^{\circ}\text{C}$ ] for the previous 24 hours, mean daily maximum temperature [ $^{\circ}\text{C}$ ] for the previous 7 days and 30 days, maximum dew point [ $^{\circ}\text{C}$ ] for the previous 24 hours, mean daily maximum dew point [ $^{\circ}\text{C}$ ] for the previous 7 days and 30 days, maximum relative humidity [%] for the previous 24 hours, mean daily maximum relative humidity [%] for the previous 7 days and 30 days, maximum wind speed [KPH] for the previous 24 hours, and mean daily maximum wind speed [KPH] for the previous 7 days and 30 days at the nearest weather station in Yankton,

South Dakota or Sioux City, Iowa), first for all chicks combined, and second, for two groups of chicks (i.e. chicks with  $CORT_{30} - CORT_B > 0$ ,  $n=9$ , and chicks with  $CORT_{30} - CORT_B \leq 0$ ,  $n=15$ ). Since 0 and negative numbers cannot be log-transformed (i.e.  $\log \leq 0$  is undefined), for the analysis of all chicks  $CORT_{30} - CORT_B \leq 0$ , CORT response to restraint stress relative to the baseline CORT was given the log-transformed value of 0, and the between-group  $CORT_{30} - CORT_B$  data was not log-transformed. In all of these analyses, where there were nests with missing data due to equipment malfunction (i.e.  $n=1$  for roof characteristics,  $n=2$  for anemometer  $T_a$  and wind speed,  $n=4$  for ovoid  $T_e$ ,  $n=15$  for iButton  $T_a$ , as indicated on Tables 2-5), we performed simple linear regression.

### **DNA Samples Collection**

Dead chicks, unhatched eggs, blood from living chicks, as well as incidental fecal and feather samples were collected from rooftop nest sites at four different urban locations in southeastern South Dakota, USA. In addition, remaining red blood cells from the corticosterone study were used. Two incidental adults found dead were collected as well. Samples were stored at  $-80^{\circ}\text{C}$  prior to DNA extraction.

Genomic DNA was extracted from all nighthawk tissue samples using the Qiagen DNeasy® Blood and Tissue kit (Qiagen, Inc., Valencia, CA). Approximately 25mg tissue samples from chicks and eggs were extracted following the DNeasy® animal tissue spin-column protocol. Chick tissue samples were digested overnight. Egg tissue samples were digested for 2-4 hours. Approximately 25mg fecal samples and feather samples were extracted following a modified DNeasy® tissue extraction protocol. Ten microliter blood samples were extracted following the DNeasy® animal blood spin-column protocol. Extracted DNA was stored in elution buffer at  $-20^{\circ}\text{C}$  prior to use for polymerase chain reaction (PCR).



DNA was amplified via PCR using primers for the mitochondrial DNA (mtDNA) *Cytochrome-b* gene region (*MT-CYB* L14764 and *MT-CYB* H16060)(Han et al. 2010) and Taq PCR master mix (Qiagen, Inc., Valencia, CA) following Han et al. (2010). PCR products were run on a 1.5% agarose gel using GelRed™ prestain loading buffer (Biotium, Inc., Fremont, CA) to confirm successful amplification. DNA extractions, PCR amplification, and gel electrophoresis were carried out in separate areas with designated equipment to prevent contamination. PCR products were cleaned in preparation for sequencing using ExoSAP-IT (Affymatrix, Inc., Cleveland, OH) to remove excess primers and nucleotides, and sent to the Arizona State University School of Life Sciences DNA Laboratory for unidirectional sequencing using the *MT-CYB* H16060 primer.

Sequence results were cleaned using FinchTV 1.4.0 (Geospiza, Inc., Seattle, WA) and aligned using ClustalW in BioEdit 7.2.5 (Hall, 1999). The single indel (sequence gap) in one sequence was coded as missing data. Sequences were compared with sequences published in GenBank for construction of phylogenetic trees. Haplotype diversity estimates, gene flow and genetic differentiation analyses, identification of synonymous and non-synonymous substitutions as well as Tajima's D test for selective neutrality were carried out in DnaSP 5.10.01 (Librado and Rozas, 2009). Nucleotide diversity ( $\pi$ ) values were calculated based upon variation at synonymous sites. The TCS haplotype network was made using Subpopulation Analysis with Reticulate Trees (PopART) software (<http://popart.otago.ac.nz>), and the statistical parsimony haplotype network was made using TCS 1.21 software (Clement et al. 2000).

### **Genetics Study Statistics**

We used a Chi-square test for differences in haplotype frequencies across the four sample sites (i.e. Vermillion, Yankton, North Sioux City and Elk Point) to estimate gene flow between four

sample sites, hereafter referred to as subpopulations. A Tajima's D test was used to indicate selective neutrality and the presence of a recent populations bottleneck (Tajima 1989).

## Results

For regional climate data for the three weather stations, we found significantly increasing mean humidity (%) and maximum wind (KPH), decreasing cloud cover (%), mean wind (KPH) and maximum gust (KPH) in two of the weather stations and decreasing maximum visibility (km), mean visibility (km) and minimum visibility (km) in all three weather stations during the local nighthawk breeding season (May 15-August 15) 1948-2016 (summarized in Table 1).

When comparing roof characteristics with microclimate dependent variables, our results showed significant relationship only for that between iButton ambient temperature ( $T_a$ ) ( $^{\circ}\text{C}$ ) and roof height (m); however this was with a small sample size ( $P<0.001$ ,  $F_{1,4}=23.59$ , Adj.  $r^2=0.819$ ,  $Coef=1.6680$ ) (Table 2). Chicks did not significantly differ within nests for any of the CORT metrics (Table 3).

For the data set including all nests combined together,  $\log\text{CORT}_B$  size significantly increased with increasing anemometer  $T_a$  ( $^{\circ}\text{C}$ ) ( $P<0.001$ ,  $F_{1,13}=22.81$ , Adj.  $r^2=0.609$ ,  $Coef=0.075$ ),  $\log\text{CORT}_{30}$  significantly decreased with increasing anemometer maximum wind (KPH) ( $P<0.05$ ,  $F_{1,13}=5.598$ , Adj.  $r^2=0.247$ ,  $Coef=-0.004$ ),  $\log\text{CORT}_B$  significantly increased with increasing ovoid  $T_e$  ( $^{\circ}\text{C}$ ) ( $P<0.05$ ,  $F_{1,12}=5.318$ , Adj.  $r^2=0.249$ ,  $Coef=0.0450$ ), and none of the temporal, roof, chick condition, chick number, other microclimate, regional climate variables were significant predictors of the CORT metrics (Table 3).

When we separated the chicks into two groups (i.e. chicks with  $\text{CORT}_{30} - \text{CORT}_B > 0$ ,  $n=9$ , and chicks with  $\text{CORT}_{30} - \text{CORT}_B \leq 0$ ,  $n=15$ ), for the  $\text{CORT}_{30} - \text{CORT}_B \leq 0$  group, we found

no significant predictors of changes in the CORT metrics (Table 4). For the  $CORT_{30} - CORT_B > 0$  group, we found some evidence (i.e. predictive strength is limited due to smaller sample sizes) that increasing iButton  $T_a$  ( $^{\circ}C$ ) was a significant predictor of decreasing  $\log CORT_{30}$  ( $P < 0.001$ ,  $F_{1,3} = 72.91$ , Adj.  $r^2 = 0.9473$ ,  $Coef = -0.3965$ ) and decreasing  $CORT_{30} - CORT_B$  ( $P < 0.01$ ,  $F_{1,13} = 1019$ , Adj.  $r^2 = 0.9963$ ,  $Coef = -19.6065$ ) (Table 5).

Of the 66 individual nighthawk samples collected, DNA from 38 were successfully amplified and sequenced (Tables 6-10). The *MT-CYB* primers amplified about 1140 base pairs (bp) for most samples. The sequences for some samples were shorter, likely due to degradation of the sample before collection. After alignment, all sequences were trimmed and a 630bp region was used for analysis. One sequence from the only successfully amplified feather sample was much shorter ( $< 500bp$ ). This sample was not included in the genetic analysis.

There was a total of 11 different haplotypes (h) present across all sampling locations, with differing numbers of haplotypes present in each location (Table 10). Overall, nucleotide diversity ( $\pi$ ) for all 37 sequences was low (0.0210). Haplotype diversity estimates the probability that two randomly selected haplotypes in a subpopulation are different. Elk Point, Vermillion, and Yankton locations had similar haplotype diversity. The North Sioux City location had slightly lower haplotype diversity (Table 10).

A Chi-square test for differences in haplotype frequencies across the 4 sample sites was significant ( $\chi^2 = 52.85$ ,  $p = 0.006$ ) indicating that haplotypes are unevenly distributed between the four sampling locations (Hudson et al. 1992). The  $F_{ST}$  values in Table 11 can be used to estimate levels of gene flow between subpopulations. Low  $F_{ST}$  values indicate low genetic differentiation and higher gene flow between subpopulations (Table 11). The most differentiated pair of sites was Elk Point and Yankton. The most similar pair of sites was Vermillion and North

Sioux City. The locations with the most gene flow between them are the locations nearest each other geographically, with lower gene flow occurring between the more widely separated locations. Tajima's D test indicates selective neutrality (Tajima's  $D = -0.40$ ,  $p > 0.1$ ) and its negative value suggests a recent population bottleneck (Tajima 1989).

The two haplotype networks present the evolutionary relationships between the haplotypes identified in this study (Figures 2 and 3). The TCS network identifies the different haplotypes and the locations at which each haplotype was found (Figure 2). The slashes across branch network lines of the TCS network indicate a nucleotide change. The size of the circle represents the number of samples of each haplotype. The pie chart colors represent the locations at which each haplotype was found. This network shows that several haplotypes were represented in multiple locations, with a few haplotypes being exclusive to one location.

The statistical parsimony network also presents the different haplotypes and the locations at which they were found (Figure 3). In this network, various colors represent the different haplotypes. The pie charts demonstrate the proportions of each haplotype found in each location. It is notable that of the four haplotypes identified in Elk Point, only one is shared with the North Sioux City location, and none of the haplotypes are shared with the Vermillion and Yankton locations; however, one of the reference sequences from GenBank is identical to the shared haplotype, indicating that it is likely a common haplotype in nighthawk subpopulations. Even more notable is the fact that very few nighthawks were found in Elk Point. All samples came from the nests of four different females, all presumed to be mating with the same male. This indicates that the four females present are unrelated to each other, and unrelated to nighthawks at any other location except North Sioux City.

Vermillion had the greatest number of haplotypes identified; likely due to the larger sample size from Vermillion. Of the six haplotypes detected in Vermillion, three were shared with the Yankton location, and one with the North Sioux City location. Yankton shared two haplotypes with North Sioux City. One of these haplotypes is identical to the second reference sample from GenBank, which interestingly was not identified in Vermillion in spite of the larger sample size.

## **Discussion**

Our genetics study results should be regarded as preliminary due to our relatively small sample sizes from a small number of locations that were available for analysis. On the other hand, we detected some subpopulation-level differences that may be important for conservation efforts for the Common Nighthawk in eastern South Dakota. These results suggest that there is little subpopulation genetic differentiation between the Yankton, Vermillion, and North Sioux City locations. In contrast, the Elk Point samples demonstrated the greatest level of genetic differentiation from the other sites and contained a nearly unique set of mtDNA haplotypes compared to the other samples despite our small sample size ( $n = 7$ ) from North Sioux City. Our results suggest that this may be an historic artifact of differential habitat use perhaps exacerbated by relatively recent subpopulation bottlenecks. Specifically, we speculate that this pattern may be due in part to a historic difference in habitat use in which the ancestral Yankton, Vermillion, North Sioux City subpopulation used primarily riparian nesting sites along the Missouri River while the ancestral Elk Point subpopulation was more likely to utilize upland nesting sites associated with the nearby Loess Hills. This potential segregation of nesting habitat may have contributed to the genetic structuring that we detected in this study. Note that the Yankton,

Vermillion, North Sioux City samples are not genetically homogeneous and that recent habitat fragmentation and subpopulation bottlenecks could have driven the lower levels of subpopulation genetic differentiation that we observed among these three sample locations. Moreover, with recent agricultural intensification, gene flow between subpopulations can occur across the relatively undisturbed riverine land cover types to a greater extent than the terrestrial land cover types that are now largely row crop agriculture.

Because Common Nighthawks nest in an open scrape on rooftops in our study area with no tree cover and are relatively exposed to weather, we examined whether the climate had changed in the study region and whether nighthawks have adapted to the changes. In the last 68 years, nighthawks in our study region have had to adapt to increasing humidity, which could affect their ability to effectively evaporate water while respiring during the hottest parts of the day, decreasing cloud cover, which could increase the radiance and thus, the temperature of the open rooftop sites, and decreasing wind and gust speeds that would otherwise mitigate the high temperatures of the rooftop sites.

To further understand the role that varying conditions at rooftop sites play in terms of temperature, it's possible, based upon our limited data's sample size, to infer that temperatures increase with higher roof heights. However, roof heights were not shown to be a predictor of any of the CORT metrics, and thus, we cannot be definitive on our conclusions regarding our rooftop metrics. If rooftops continue to be a nesting habitat for nighthawks, and there are added challenges to this habitat to address, including urban predators and declining use of gravel as a flat rooftop substrate, more research is needed to determine optimal rooftop characteristics with parapet height, rooftop height, and gravel diameter being some of the few metrics to study.

Nonetheless, our results show some evidence that nighthawk chick baseline corticosterone levels increase with higher anemometer ( $T_a$ ) and ovoid ( $T_e$ ) temperatures, as shown in our data for all nests combined. This suggests that nighthawk chick condition might be compromised by the urban “heat island” conditions of rooftop nesting sites, but without more corroboration between corticosterone metrics and other microclimate and regional climate conditions, it’s difficult to be more certain. Based upon our observations of notable chick die-offs after heat waves, thunderstorms and their accompanying higher humidity levels (our unpublished work), we expected but did not find an association between regional climate conditions on the day and week scale and higher baseline corticosterone and lower baseline corticosterone relative to stressed-induced corticosterone, suggesting the majority of nighthawks are capable of withstanding heat stress on a small time scale.

In addition, we separated the chicks into two groups. First was a group that showed elevated baseline corticosterone relative to stressed-induced corticosterone (i.e.  $CORT_{30} - CORT_B \leq 0$ ) and thus, a lowered ability to respond to handling stress. Since this group ( $n=16$ ) outnumbered the second group with its low baseline corticosterone relative to stressed-induced corticosterone (i.e.  $CORT_{30} - CORT_B > 0$ ) ( $n=6$ ), our results suggest the majority of our study’s population is experiencing a compromised ability to cope with the changed climatic conditions. However, due to small sample size, it is our conservative opinion that the association between increasing iButton  $T_a$  ( $^{\circ}C$ ) and decreasing  $\log CORT_{30}$  and decreasing  $CORT_{30} - CORT_B$  requires further study to be more certain with our assertions.

This suggests that urban rooftops might be suitable habitat for Common Nighthawks in the Northern Prairie region, provided predation rates remain low and that gravel remains a predominant material for flat rooftops. Urban areas are subject to the increasing prevalence of

nestling and fledgling predation by crows and other species, which has likely contributed to declines of urban populations of nighthawks in North America (Marzilli 1989, Wedgwood 1991, COSEWIC 2007, Latta and Latta 2015). Conversion of gravel rooftops to other materials is a trend in both Canada and the U.S. (Brigham et al. 2011) and in our study area in southeastern South Dakota (our unpublished work), so as flat, gravel rooftops are replaced in a 7-10 year interval, we can infer that suitable urban nesting sites will likely rapidly decline throughout North America by 2025 (our unpublished work).

Our results suggest our study region's nighthawks have adapted to current conditions as a result of recent climate change. However, climate change might reach a threshold in the future that would affect chick condition. While nighthawks and other nightjars in arid conditions and as adults have been shown to be adaptable to extremely high temperatures (Cowles and Dawson 1951, Howell 1959, Bartholomew et al. 1962, Lasiewski and Dawson 1964, O'Connor et al. 2016), rising humidity for chicks in temperate areas like the Northern Plains could affect evaporative water loss (EWL) rates to a greater extent due to chicks' smaller and undeveloped gular regions and trigger an elevated baseline corticosterone response, especially in younger chicks than our study's 14-day-old chicks. Moreover, the Great Plains region is expected to have increasing temperatures and dew points by the year 2100 (Kenward et al. 2014). More research is needed to find the temperature and humidity thresholds that induce heat stress in Common Nighthawk chicks to determine whether these climate change scenarios will affect chick baseline corticosterone levels and ultimately, their survival.

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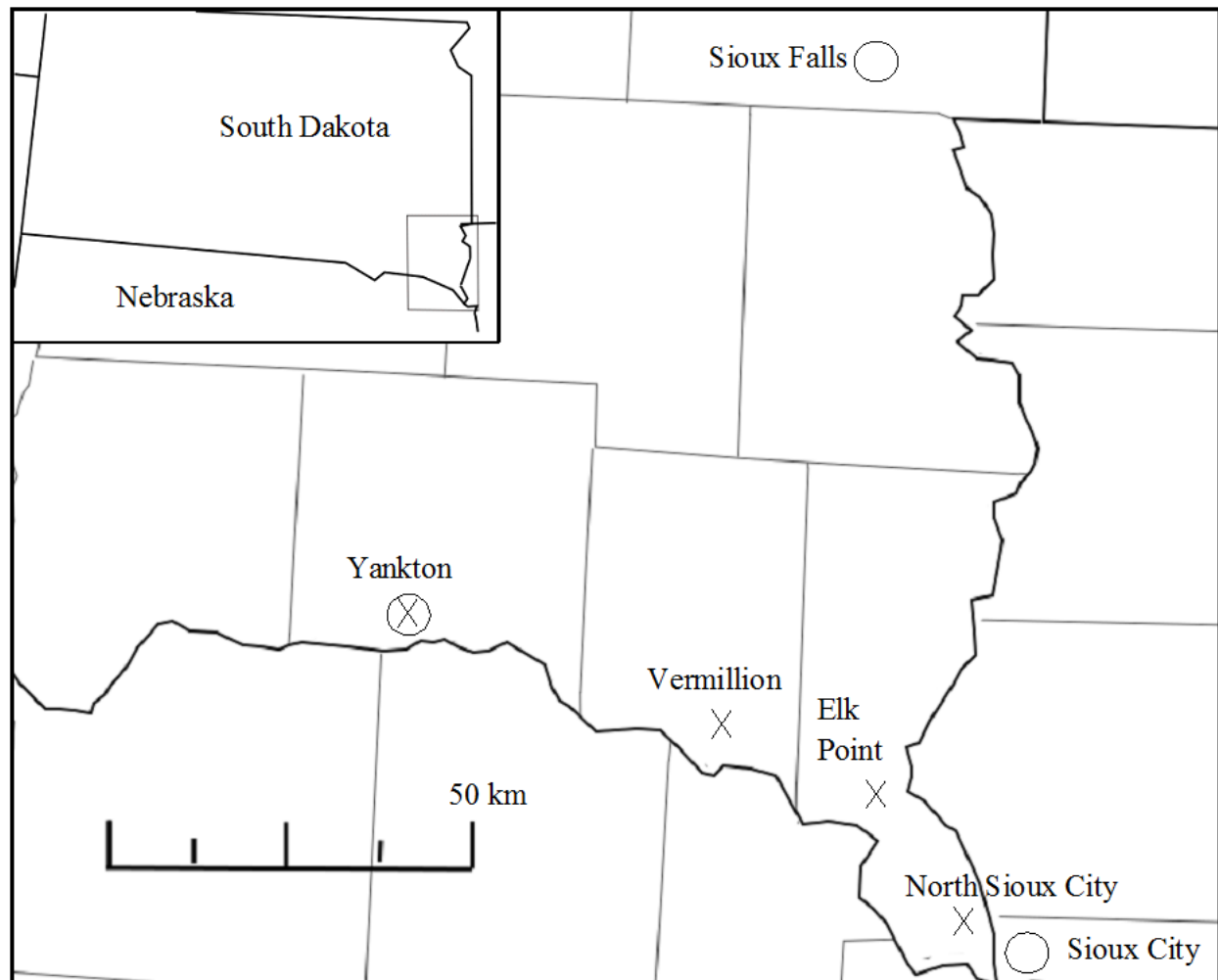
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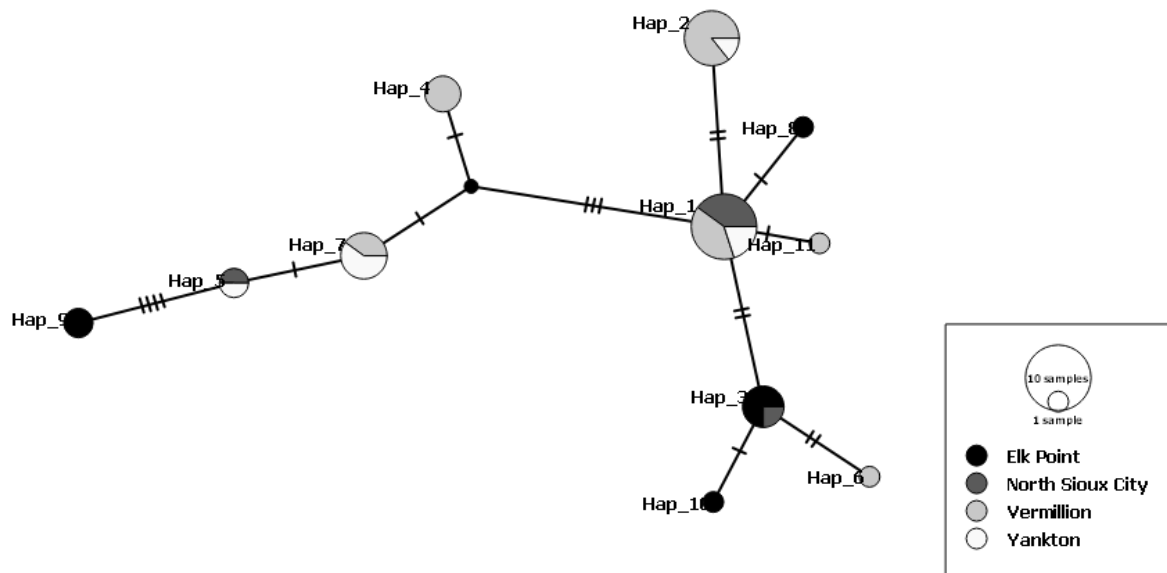
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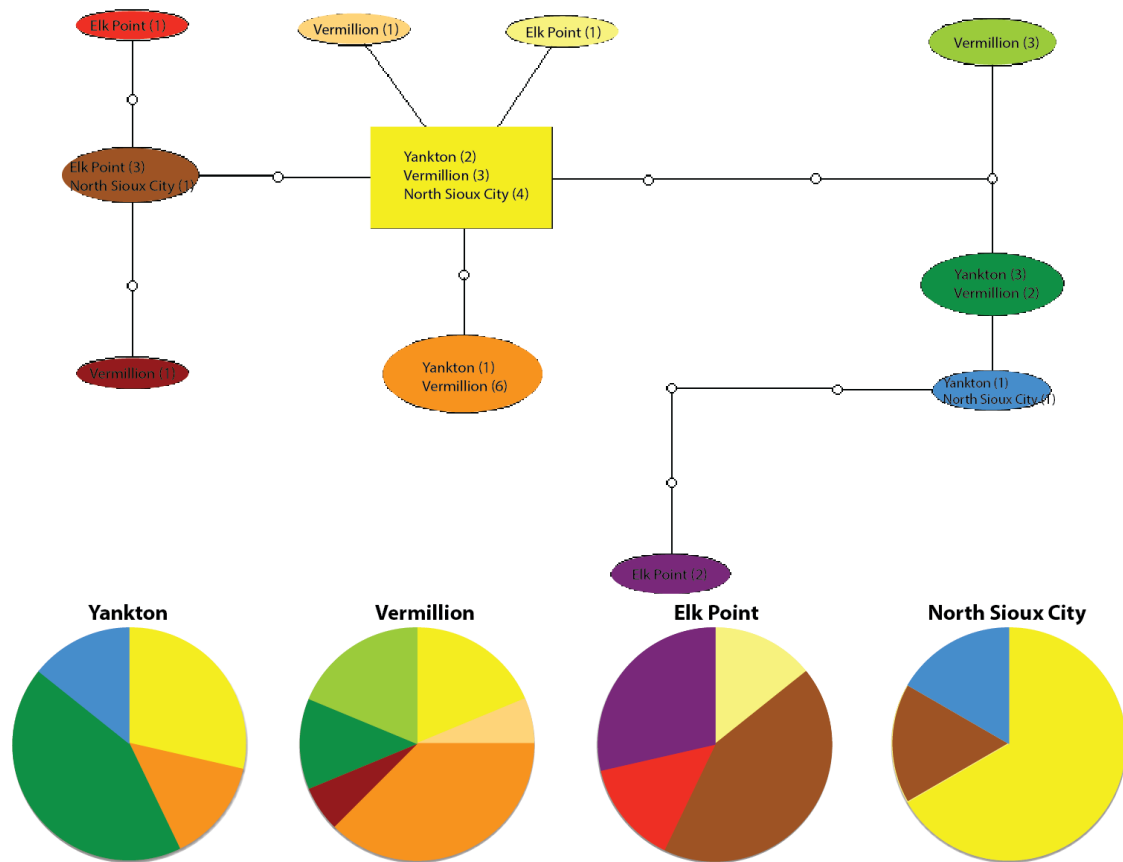
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**FIGURE 1:** Cities with rooftop nest study sites (i.e. X) and NOAA weather stations (i.e. Circles) in South Dakota and Iowa.



**FIGURE 2:** TCS network based on 1,000 iterations. Size of circles represent number of samples of each haplotype (Hap\_n) and colors represent sample locations for each haplotype. Slashes across branch network lines represents a nucleotide change between haplotypes.



**FIGURE 3:** Statistical parsimony haplotype network. Colored shapes represent each haplotype. The locations at which each haplotype were found and the number of each haplotype by location are noted within each haplotype shape. The pie charts below demonstrate the proportion of haplotypes found at each location. Circles within the branch network lines represent nucleotide changes between haplotypes.



**TABLE 1.** Summary of regression analyses of interval means of daily summer (May 15-August 15, 1948-2016) environmental data (as dependent variables) from three surrounding weather stations (Yankton, SD, Sioux City, IA, and Sioux Falls, SD) with year as a predictor variable. For each year, dependent variable with significant p-values ( $\alpha < 0.05$ ) is expected to change by coefficient amount. Years missing data are reflected in *df*. Adjusted  $r^2$  values are reported. Dependent variables with  $P < 0.05$  at same direction of coefficient at all three weather stations are denoted with \*. Dependent variables with  $P < 0.05$  at same direction of coefficient at two weather stations are denoted with \*\*.

Station	Dependent Variable	<i>F</i>	<i>df</i>	Adj $r^2$	<i>P</i>	<i>Coef</i>
Yankton	Maximum Temp (°C)	6.04	1,67	0.069	<0.05	-0.022
	Mean Temp (°C)	0.5109	1,66	-0.007	0.48	0.007
	Minimum Temp (°C)	4.204	1,67	0.045	<0.05	0.033
	Maximum Dew Point (°C)	0.0095	1,42	-0.024	0.92	-0.001
	Mean Dew Point (°C)	1.55	1,42	0.013	0.22	-0.024
	Minimum Dew Point (°C)	1.84	1,42	0.019	0.18	-0.033
	Maximum Humidity (%)	14.42	1,42	0.238	<0.001	0.238
	Mean Humidity ( %)	13.13	1,42	0.22	<0.001**	0.224
	Minimum Humidity (%)	1.048	1,42	0.001	0.31	-0.070
	Maximum Visibility (km)	14.31	1,42	0.236	<0.001*	-0.096
	Mean Visibility (km)	5.894	1,42	0.102	<0.05 *	-0.056
	Minimum Visibility (km)	26.64	1,42	0.374	<0.001*	-0.132
	Maximum Wind (KPH)	9.305	1,42	0.162	<0.05**	0.139
	Mean Wind (KPH)	0.410	1,42	-0.014	0.53	-0.013
	Maximum Gust (KPH)	1.484	1,42	0.011	0.23	-0.050
	Precipitation (cm)	0.458	1,52	-0.010	0.50	0.010
	Cloud Cover (%)	112.6	1,42	0.722	<0.001**	-0.091
Sioux City	Maximum Temp (°C)	0.363	1,70	-0.009	0.55	0.005
	Mean Temp (°C)	0.1168	1,70	-0.013	0.73	-0.001
	Minimum Temp (°C)	4.402	1,70	0.0457	<0.05	-0.010
	Maximum Dew Point (°C)	1.928	1,70	0.013	0.169	0.007
	Mean Dew Point (°C)	2.085	1,70	0.015	0.15	0.008
	Minimum Dew Point (°C)	1.96	1,70	0.013	0.17	0.008
	Maximum Humidity (%)	2.614	1,70	0.022	0.11	0.036
	Mean Humidity ( %)	3.679	1,70	0.036	0.06	0.045
	Minimum Humidity (%)	1.463	1,70	0.006	0.23	0.030
	Maximum Visibility (km)	5.977	1,70	0.066	<0.05*	-0.083

1	Mean Visibility (km)	9.436	1,70	0.106	<0.001*	-0.073
	Minimum Visibility (km)	19.08	1,70	0.203	<0.001*	-0.071
	Maximum Wind (KPH)	3.901	1,70	0.039	0.052	0.033
	Mean Wind (KPH)	11.61	1,70	0.13	<0.001**	-0.030
	Maximum Gust (KPH)	38.22	1,42	0.464	<0.001**	-0.188
	Precipitation (cm)	2.632	1,68	0.023	0.109	-0.011
	Cloud Cover (%)	7.817	1,44	0.132	<0.001**	-0.026
Sioux Falls	Maximum Temp (°C)	0.041	1,70	-0.014	0.84	0.002
	Mean Temp (°C)	0.080	1,70	-0.013	0.78	0.002
	Minimum Temp (°C)	0.104	1,70	-0.013	0.75	0.002
	Maximum Dew Point (°C)	7.195	1,70	0.080	<0.001	0.015
	Mean Dew Point (°C)	11.71	1,70	0.131	<0.001	0.020
	Minimum Dew Point (°C)	15.18	1,70	0.167	<0.001	0.025
	Maximum Humidity (%)	3.113	1,70	0.029	0.08	0.039
	Mean Humidity (%)	5.499	1,70	0.060	<0.05**	0.062
	Minimum Humidity (%)	7.096	1,70	0.080	<0.001	0.075
	Maximum Visibility (km)	23.65	1,70	0.242	<0.001*	-0.102
	Mean Visibility (km)	21.86	1,70	0.227	<0.001*	-0.080
	Minimum Visibility (km)	15.72	1,70	0.172	<0.001*	-0.053
	Maximum Wind (KPH)	4.115	1,70	0.042	<0.05**	0.029
	Mean Wind (KPH)	13.19	1,70	0.147	<0.001**	-0.026
	Maximum Gust (KPH)	19.32	1,42	0.299	<0.001**	-0.096
	Precipitation (cm)	0.010	1,67	-0.015	0.922	-0.200
	Cloud Cover (%)	0.060	1,46	-0.020	0.81	0.002

**TABLE 2.** Summary of regression analyses of microclimate dependent variables with roof characteristics (as predictor variables). For each increment of predictor variable, dependent variable with significant p-values ( $\alpha < 0.05$ ) is expected to change by coefficient amount. All measurements are at time of blood collection. Nests missing data are reflected in *df*. Adjusted  $r^2$  values are reported. Where there were nests with missing data (i.e. n=1 for roof characteristics, n=2 for anemometer  $T_a$  and wind speed, n=4 for ovoid  $T_e$ , n=15 for iButton  $T_a$ , as denoted as <sup>S</sup>), we performed simple linear regression. Dependent variables with  $P < 0.05$  at same direction of coefficient at all three weather stations are denoted with \*. Dependent variables with  $P < 0.05$  at same direction of coefficient at two weather stations are denoted with \*\*.

Predictor Variable	Dependent Variable	<i>F</i>	<i>df</i>	Adj $r^2$	<i>P</i>	<i>Coef</i>
Parapet Height (cm) <sup>S</sup>	Anemometer $T_a$ (°C)	0.1093	1,12	-0.074	0.75	0.0431
	Anemometer Max Wind (KPH)	2.291	1,12	0.090	0.16	1.7910
	Ovoid $T_e$ (°C)	0.5369	1,12	-0.037	0.48	0.1333
	iButton $T_a$ (°C)	0.0239	1,4	-0.243	0.88	0.0620
Roof Height (m) <sup>S</sup>	Anemometer $T_a$ (°C)	0.0217	1,12	-0.081	0.89	-0.071
	Anemometer Max Wind (KPH)	0.3430	1,12	-0.053	0.57	-2.766
	Ovoid $T_e$ (°C)	1.631	1,12	0.046	0.23	0.6972
	iButton $T_a$ (°C)	23.59	1,4	0.819	<0.001**	1.6680
Mean Gravel Diameter (cm) <sup>S</sup>	Anemometer $T_a$ (°C)	0.7808	1,12	-0.017	0.39	0.3641
	Anemometer Max Wind (KPH)	0.0219	1,12	-0.081	0.89	0.6184
	Ovoid $T_e$ (°C)	0.0002	1,12	-0.083	0.99	0.0062
	iButton $T_a$ (°C)	0.0116	1,4	-0.246	0.92	-0.0943

**TABLE 3.** Summary of regression analyses of logCORT<sub>B</sub>, logCORT<sub>30</sub>, and logCORT<sub>30</sub>-logCORT<sub>B</sub> (as dependent variables) with temporal, roof, chick condition, microclimate and regional climate predictor variables of **all chicks combined**. For each increment of predictor variable, dependent variable with significant p-values ( $\alpha < 0.05$ ) is expected to change by coefficient amount. All measurements are at time of blood collection, except for regional climate specified intervals. Nests missing data are reflected in *df*. Adjusted  $r^2$  values are reported. Since 0 and negative numbers cannot be log-transformed (i.e.  $\log \leq 0$  is undefined), for the analysis of all chicks  $\text{CORT}_{30} - \text{CORT}_B \leq 0$  was given the log-transformed value of 0. Where there were nests with missing data (i.e. n=1 for roof characteristics, n=2 for anemometer T<sub>a</sub> and wind speed, n=4 for ovoid T<sub>e</sub>, n=15 for iButton T<sub>a</sub>, as denoted as <sup>S</sup>), we performed simple linear regression.

Predictor Variable Model	Predictor Variable	Dependent Variable	<i>F</i>	<i>df</i>	Adj $r^2$	<i>P</i>	<i>Coef</i>
Temporal	Year	logCORT <sub>B</sub>	0.4194	3,20	-0.082	0.94	-1.198
		logCORT <sub>30</sub>	0.3947	3,13	-0.128	0.37	-0.136
		logCORT <sub>30</sub> -logCORT <sub>B</sub>	1.367	3,13	0.064	0.11	-20.16
	Julian Date with Year	logCORT <sub>B</sub>	0.4194	3,20	-0.082	0.96	0.0007
		logCORT <sub>30</sub>	0.3947	3,13	-0.128	0.37	0.0138
		logCORT <sub>30</sub> -logCORT <sub>B</sub>	1.367	3,13	0.064	0.11	0.0219
	Julian Date	logCORT <sub>B</sub>	0.076	1,22	-0.042	0.79	-0.0038
		logCORT <sub>30</sub>	1.074	1,15	0.005	0.32	0.0124
		logCORT <sub>30</sub> -logCORT <sub>B</sub>	2.899	1,22	0.076	0.10	0.0208
	Decimal Time	logCORT <sub>B</sub>	0.4194	3,20	-0.082	0.91	-0.0093
		logCORT <sub>30</sub>	0.3947	3,13	-0.128	0.65	0.0347
		logCORT <sub>30</sub> -logCORT <sub>B</sub>	1.367	3,13	0.064	0.42	0.0531
Roof	Parapet Height	logCORT <sub>B</sub>	0.8729	3,19	-0.018	0.23	0.0150
		logCORT <sub>30</sub>	0.386	3,12	-0.14	0.60	0.0060

Characteristics	(cm) <sup>S</sup>	logCORT <sub>30</sub> - logCORT <sub>B</sub>	1.048	3,12	0.010	0.63	-0.050
		logCORT <sub>B</sub>	0.8729	3,19	-0.018	0.58	0.0280
		logCORT <sub>30</sub>	0.386	3,12	-0.14	0.68	0.0189
	Roof Height (m) <sup>S</sup>	logCORT <sub>30</sub> - logCORT <sub>B</sub>	1.048	3,12	0.010	0.92	0.0037
		logCORT <sub>B</sub>	0.8729	3,19	-0.018	0.40	0.038
		logCORT <sub>30</sub>	0.386	3,12	-0.14	0.47	-0.029
	Mean Gravel Diameter (cm) <sup>S</sup>	logCORT <sub>30</sub> - logCORT <sub>B</sub>	1.048	3,12	0.010	0.19	-0.046
		logCORT <sub>B</sub>	0.8729	3,19	-0.018	0.40	0.038
		logCORT <sub>30</sub>	0.386	3,12	-0.14	0.47	-0.029
	Chick Condition	logCORT <sub>B</sub>	0.7141	2,21	-0.025	0.69	-0.005
		logCORT <sub>30</sub>	0.2601	2,14	-0.102	0.57	0.006
		logCORT <sub>30</sub> - logCORT <sub>B</sub>	0.6506	2,14	-0.046	0.55	0.006
		logCORT <sub>B</sub>	0.7141	2,21	-0.025	0.35	0.007
		logCORT <sub>30</sub>	0.2601	2,14	-0.102	0.80	-0.002
		logCORT <sub>30</sub> - logCORT <sub>B</sub>	0.6506	2,14	-0.046	0.45	-0.005
Microclimate	Anemometer T <sub>a</sub> (°C) <sup>S</sup>	logCORT <sub>B</sub>	22.81	1,13	0.609	<0.001	0.075
		logCORT <sub>30</sub>	1.377	1,13	0.026	0.26	0.023
		logCORT <sub>30</sub> - logCORT <sub>B</sub>	0.2197	1,13	-0.060	0.65	-0.008
	Anemometer Max Wind (KPH) <sup>S</sup>	logCORT <sub>B</sub>	1.7200	1,13	0.049	0.21	-0.003
		logCORT <sub>30</sub>	5.598	1,13	0.247	<0.05	-0.004
		logCORT <sub>30</sub> - logCORT <sub>B</sub>	0.2313	1,13	-0.058	0.64	-0.001
	Ovoid T <sub>e</sub> (°C) <sup>S</sup>	logCORT <sub>B</sub>	5.318	1,12	0.249	<0.05	0.0450
		logCORT <sub>30</sub>	0.0937	1,12	-0.079	0.77	-0.0062
		logCORT <sub>30</sub> - logCORT <sub>B</sub>	1.545	1,12	0.040	0.24	-0.0235
	iButton T <sub>a</sub> (°C) <sup>S</sup>	logCORT <sub>B</sub>	0.8860	1,4	-0.023	0.40	0.0295
		logCORT <sub>30</sub>	0.7235	1,4	-0.058	0.44	-0.0219
		logCORT <sub>30</sub> - logCORT <sub>B</sub>	0.8490	1,4	-0.031	0.41	-0.0301
Regional Climate	Maximum T <sub>a</sub> (°C) for Day	logCORT <sub>B</sub>	2.930	4,12	0.592	0.68	0.0691
		logCORT <sub>30</sub>	0.487	4,12	-0.626	0.29	0.3126
		logCORT <sub>30</sub> - logCORT <sub>B</sub>	1.274	4,12	0.170	0.12	0.3441
		logCORT <sub>B</sub>					

Maximum T <sub>a</sub> (°C) for Week	logCORT <sub>B</sub>	2.930	4,12	0.592	0.11	0.6022
	logCORT <sub>30</sub>	0.487	4,12	-0.626	0.57	0.3280
	logCORT <sub>30</sub> -	1.274	4,12	0.170	0.23	0.5105
	logCORT <sub>B</sub>					
Maximum T <sub>a</sub> (°C) for 30 Days	logCORT <sub>B</sub>	2.930	4,12	0.592	0.06	3.2130
	logCORT <sub>30</sub>	0.487	4,12	-0.626	0.26	2.8840
	logCORT <sub>30</sub> -	1.274	4,12	0.170	0.29	1.7939
	logCORT <sub>B</sub>					
Maximum Dew Point (°C) for Day	logCORT <sub>B</sub>	2.930	4,12	0.592	0.05	-0.4024
	logCORT <sub>30</sub>	0.487	4,12	-0.626	0.23	-0.3809
	logCORT <sub>30</sub> -	1.274	4,12	0.170	0.10	-0.3846
	logCORT <sub>B</sub>					
Maximum Dew Point (°C) for Week	logCORT <sub>B</sub>	2.930	4,12	0.592	0.08	-0.8820
	logCORT <sub>30</sub>	0.487	4,12	-0.626	0.33	-0.7769
	logCORT <sub>30</sub> -	1.274	4,12	0.170	0.13	-0.8886
	logCORT <sub>B</sub>					
Maximum Dew Point (°C) for 30 Days	logCORT <sub>B</sub>	2.930	4,12	0.592	0.09	-2.2200
	logCORT <sub>30</sub>	0.487	4,12	-0.626	0.30	-2.1710
	logCORT <sub>30</sub> -	1.274	4,12	0.170	0.36	-1.2694
	logCORT <sub>B</sub>					
Maximum Humidity ( %) for Day	logCORT <sub>B</sub>	2.930	4,12	0.592	0.14	0.2960
	logCORT <sub>30</sub>	0.487	4,12	-0.626	0.46	0.2383
	logCORT <sub>30</sub> -	1.274	4,12	0.170	0.23	0.2772
	logCORT <sub>B</sub>					
Maximum Humidity ( %) for Week	logCORT <sub>B</sub>	2.930	4,12	0.592	0.32	-0.2603
	logCORT <sub>30</sub>	0.487	4,12	-0.626	0.75	-0.1437
	logCORT <sub>30</sub> -	1.274	4,12	0.170	0.58	-0.1679
	logCORT <sub>B</sub>					
Maximum Humidity ( %) for 30 Days	logCORT <sub>B</sub>	2.930	4,12	0.592	0.06	0.9125
	logCORT <sub>30</sub>	0.487	4,12	-0.626	0.16	1.1150
	logCORT <sub>30</sub> -	1.274	4,12	0.170	0.12	0.8605
	logCORT <sub>B</sub>					
Maximum Wind (KPH) for Day	logCORT <sub>B</sub>	2.930	4,12	0.592	0.90	-0.0057
	logCORT <sub>30</sub>	0.487	4,12	-0.626	0.61	-0.0421
	logCORT <sub>30</sub> -	1.274	4,12	0.170	0.27	-0.0681
	logCORT <sub>B</sub>					
Maximum Wind	logCORT <sub>B</sub>	2.930	4,12	0.592	0.09	0.0310
	logCORT <sub>30</sub>	0.487	4,12	-0.626	0.99	-0.0020

	(KPH) for Week	logCORT <sub>30</sub> - logCORT <sub>B</sub>	1.274	4,12	0.170	0.31	-0.2013
	Maximum Wind	logCORT <sub>B</sub>	2.930	4,12	0.592	0.05	-0.8403
	(KPH) for 30	logCORT <sub>30</sub>	0.487	4,12	-0.626	0.28	-0.6961
	Days	logCORT <sub>30</sub> - logCORT <sub>B</sub>	1.274	4,12	0.170	0.22	-0.5480
Other	Chick Number (Within Nest)	logCORT <sub>B</sub>	0.031	1,22	-0.044	0.86	-0.0043
		logCORT <sub>30</sub>	0.090	1,22	-0.041	0.77	0.0067
		logCORT <sub>30</sub> -	0.005	1,22	-0.045	0.94	-0.0016
		logCORT <sub>B</sub>					

**TABLE 4.** Summary of regression analyses of  $\log\text{CORT}_B$ ,  $\log\text{CORT}_{30}$ , and  $\text{CORT}_{30} - \text{CORT}_B$  (as dependent variables) for chicks with  $\text{CORT}_{30} - \text{CORT}_B \leq 0$  (n=15) with temporal, roof, chick condition, microclimate and regional climate predictor variables. For each increment of predictor variable, dependent variable with significant p-values ( $\alpha < 0.05$ ) is expected to change by coefficient amount. All measurements are at time of blood collection, except for regional climate specified intervals. Nests missing data are reflected in *df*. Adjusted  $r^2$  values are reported. Since 0 and negative numbers cannot be log-transformed (i.e.  $\log \leq 0$  is undefined),  $\text{CORT}_{30} - \text{CORT}_B$  data was not log-transformed. Where there were nests with missing data (i.e. n=1 for roof characteristics, n=2 for anemometer  $T_a$  and wind speed, n=4 for ovoid  $T_e$ , n=15 for iButton  $T_a$ , as denoted as <sup>S</sup>), we performed simple linear regression.

Predictor Variable Model	Predictor Variable	Dependent Variable	<i>F</i>	<i>df</i>	Adj $r^2$	<i>P</i>	<i>Coef</i>
Temporal	Year	$\log\text{CORT}_B$	1.073	3,6	0.024	0.15	78.5900
		$\log\text{CORT}_{30}$	1.008	3,6	0.003	0.20	63.13
		$\text{CORT}_{30} - \text{CORT}_B$	0.479	3,6	-0.21	0.34	-277.3
	Julian Date with Year	$\log\text{CORT}_B$	1.073	3,6	0.024	0.15	-0.0752
		$\log\text{CORT}_{30}$	1.008	3,6	0.003	0.20	-.06.23
		$\text{CORT}_{30} - \text{CORT}_B$	0.479	3,6	-0.21	0.34	2.754
	Julian Date	$\log\text{CORT}_B$	0.8466	1,8	-0.017	0.38	-0.0343
		$\log\text{CORT}_{30}$	0.310	1,8	-0.083	0.59	-0.020
		$\text{CORT}_{30} - \text{CORT}_B$	1.487	1,8	0.051	0.26	2.229
	Decimal Time	$\log\text{CORT}_B$	1.073	3,6	0.024	0.76	0.032
		$\log\text{CORT}_{30}$	1.008	3,6	0.003	0.66	-0.043
		$\text{CORT}_{30} - \text{CORT}_B$	0.479	3,6	-0.21	0.70	-2.266
Roof Characteristics	Parapet Height (cm) <sup>S</sup>	$\log\text{CORT}_B$	0.1563	3,6	-0.391	0.81	0.005
		$\log\text{CORT}_{30}$	0.1224	3,6	-0.414	0.67	0.009



		CORT <sub>30</sub> - CORT <sub>B</sub>	0.0255	3,6	-0.481	0.89	0.158
	Roof Height (m) <sup>S</sup>	logCORT <sub>B</sub>	0.1563	3,6	-0.391	0.65	0.038
		logCORT <sub>30</sub>	0.1224	3,6	-0.414	0.94	0.006
		CORT <sub>30</sub> - CORT <sub>B</sub>	0.0255	3,6	-0.481	0.98	0.126
	Mean Gravel Diameter (cm) <sup>S</sup>	logCORT <sub>B</sub>	0.1563	3,6	-0.391	0.72	0.110
		logCORT <sub>30</sub>	0.1224	3,6	-0.414	0.86	0.049
		CORT <sub>30</sub> - CORT <sub>B</sub>	0.0255	3,6	-0.481	0.84	-3.167
	Chick Condition	logCORT <sub>B</sub>	0.3477	2,7	-0.170	0.91	-0.002
		logCORT <sub>30</sub>	0.7664	2,7	-0.055	0.31	0.019
		CORT <sub>30</sub> - CORT <sub>B</sub>	0.7398	2,7	-0.061	0.30	1.112
Microclimate	Wing Length (cm)	logCORT <sub>B</sub>	0.3477	2,7	-0.170	0.43	-0.009
		logCORT <sub>30</sub>	0.7664	2,7	-0.055	0.63	-0.005
		CORT <sub>30</sub> - CORT <sub>B</sub>	0.739	2,7	-0.061	0.63	0.268
	Anemometer T <sub>a</sub> (°C) <sup>S</sup>	logCORT <sub>B</sub>	0.2626	1,7	-0.102	0.62	0.0200
		logCORT <sub>30</sub>	0.0305	1,7	-0.138	0.87	-0.0064
		CORT <sub>30</sub> - CORT <sub>B</sub>	1.2	1,7	0.024	0.31	-2.020
	Anemometer Max Wind (KPH) <sup>S</sup>	logCORT <sub>B</sub>	2.099	1,7	0.112	0.20	-0.0044
		logCORT <sub>30</sub>	2.175	1,7	0.128	0.18	-0.0042
		CORT <sub>30</sub> - CORT <sub>B</sub>	0.1954	1,7	-0.112	0.67	0.0774
	Ovoid T <sub>e</sub> (°C) <sup>S</sup>	logCORT <sub>B</sub>	0.7039	1,6	-0.044	0.43	0.0227
		logCORT <sub>30</sub>	0.0631	1,6	-0.155	0.81	-0.0071
		CORT <sub>30</sub> - CORT <sub>B</sub>	0.9811	1,6	-0.003	0.36	-1.494
	iButton T <sub>a</sub> (°C) <sup>S</sup>	logCORT <sub>B</sub>	0.0154	1,1	-0.970	0.92	0.0465
		logCORT <sub>30</sub>	0.5285	1,1	-0.309	0.60	-0.0336
		CORT <sub>30</sub> - CORT <sub>B</sub>	0.0039	1,1	-0.992	0.96	0.0763
Regional Climate	Maximum T <sub>a</sub> (°C) for Day	logCORT <sub>B</sub>	11.8	8,1	0.906	0.40	8.54
		logCORT <sub>30</sub>	0.7789	8,1	-0.245	0.91	-2.97
		CORT <sub>30</sub> - CORT <sub>B</sub>	0.1718	8,1	-2.791	0.94	-188.11

Maximum T <sub>a</sub> (°C) for Week	logCORT <sub>B</sub>	11.8	8,1	0.906	0.40	-34.51
	logCORT <sub>30</sub>	0.7789	8,1	-0.245	0.91	11.47
	CORT <sub>30</sub> - CORT <sub>B</sub>	0.1718	8,1	-2.791	0.94	741.31
Maximum T <sub>a</sub> (°C) for 30 Days	logCORT <sub>B</sub>	11.8	8,1	0.906	0.40	124.28
	logCORT <sub>30</sub>	0.7789	8,1	-0.245	0.91	-44.09
	CORT <sub>30</sub> - CORT <sub>B</sub>	0.1718	8,1	-2.791	0.94	-2659.33
Maximum Dew Point (°C) for Day	logCORT <sub>B</sub>	11.8	8,1	0.906	0.41	-79.95
	logCORT <sub>30</sub>	0.7789	8,1	-0.245	0.91	28.91
	CORT <sub>30</sub> - CORT <sub>B</sub>	0.1718	8,1	-2.791	0.94	1717.36
Maximum Dew Point (°C) for Week	logCORT <sub>B</sub>	11.8	8,1	0.906	0.41	-557.02
	logCORT <sub>30</sub>	0.7789	8,1	-0.245	0.91	201.95
	CORT <sub>30</sub> - CORT <sub>B</sub>	0.1718	8,1	-2.791	0.94	11934.45
Maximum Dew Point (°C) for 30 Days	logCORT <sub>B</sub>	11.8	8,1	0.906	0.40	-37.59
	logCORT <sub>30</sub>	0.7789	8,1	-0.245	0.91	12.48
	CORT <sub>30</sub> - CORT <sub>B</sub>	0.1718	8,1	-2.791	0.94	810.52
Maximum Humidity ( %) for Day	logCORT <sub>B</sub>	11.8	8,1	0.906	0.41	-1.06
	logCORT <sub>30</sub>	0.7789	8,1	-0.245	0.93	0.27
	CORT <sub>30</sub> - CORT <sub>B</sub>	0.1718	8,1	-2.791	0.95	19.95
Maximum Humidity ( %) for Week	logCORT <sub>B</sub>	11.8	8,1	0.906	0.40	-65.96
	logCORT <sub>30</sub>	0.7789	8,1	-0.245	0.91	23.33
	CORT <sub>30</sub> - CORT <sub>B</sub>	0.1718	8,1	-2.791	0.94	1420.79
Maximum Humidity ( %) for 30 Days	logCORT <sub>B</sub>	1.587	1,8	0.061	0.24	-0.200
	logCORT <sub>30</sub>	2.479	1,8	0.141	0.15	-0.218
	CORT <sub>30</sub> - CORT <sub>B</sub>	0.005	1,8	-0.124	0.95	-0.593
Maximum Wind (KPH) for Day	logCORT <sub>B</sub>	0.604	1,8	-0.046	0.46	-0.025
	logCORT <sub>30</sub>	0.562	1,8	-0.051	0.48	-0.023
	CORT <sub>30</sub> - CORT <sub>B</sub>	0.239	1,8	-0.092	0.64	0.830
Maximum Wind (KPH) for Week	logCORT <sub>B</sub>	2.111	1,8	0.1099	0.18	0.114
	logCORT <sub>30</sub>	0.270	1,8	-0.088	0.62	0.041

	$CORT_{30-}$ $CORT_B$	4.012	1,8	0.251	0.08	-7.30
Maximum Wind (KPH) for 30 Days	$\log CORT_B$	0.257	1,8	-0.090	0.63	0.053
	$\log CORT_{30}$	0.047	1,8	-0.119	0.83	0.021
	$CORT_{30-}$ $CORT_B$	0.323	1,8	-0.081	0.59	-3.021

**TABLE 5.** Summary of regression analyses of  $\log\text{CORT}_B$ ,  $\log\text{CORT}_{30}$ , and  $\text{CORT}_{30} - \text{CORT}_B$  (as dependent variables) for chicks with  $\text{CORT}_{30} - \text{CORT}_B > 0$  (n=9) with temporal, roof, chick condition, microclimate and regional climate predictor variables. For each increment of predictor variable, dependent variable with significant p-values ( $\alpha < 0.05$ ) is expected to change by coefficient amount. All measurements are at time of blood collection, except for regional climate specified intervals. Nests missing data are reflected in *df*. Adjusted  $r^2$  values are reported. Since 0 and negative numbers cannot be log-transformed, for the analysis of all chicks,  $\text{CORT}_{30} - \text{CORT}_B \leq 0$  was given the log-transformed value of 0, and the between-group  $\text{CORT}_{30} - \text{CORT}_B$  data was not log-transformed. Since 0 and negative numbers cannot be log-transformed (i.e.  $\log \leq 0$  is undefined),  $\text{CORT}_{30} - \text{CORT}_B$  data was not log-transformed. Where there were nests with missing data (i.e. n=1 for roof characteristics, n=2 for anemometer  $T_a$  and wind speed, n=4 for ovoid  $T_e$ , n=15 for iButton  $T_a$ , as denoted as  $S_j$ ), we performed simple linear regression. Dependent variables with  $P < 0.05$  at same direction of coefficient at all three weather stations are denoted with \*. Dependent variables with  $P < 0.05$  at same direction of coefficient at two weather stations are denoted with \*\*.

Predictor Variable Model	Predictor Variable	Dependent Variable	<i>F</i>	<i>df</i>	Adj $r^2$	<i>P</i>	<i>Coef</i>
Temporal	Year	$\log\text{CORT}_B$	2.306	2,10	0.2316	0.24	-0.2209
		$\log\text{CORT}_{30}$	2.383	3,10	0.2419	0.07	-30.0900
		$\text{CORT}_{30} - \text{CORT}_B$	0.822	3,10	-0.0452	0.32	-0.0013
	Julian Date with Year	$\log\text{CORT}_B$	2.306	2,10	0.2316	0.26	0.0212
		$\log\text{CORT}_{30}$	2.383	3,10	0.2419	0.07	0.0301
		$\text{CORT}_{30} - \text{CORT}_B$	0.822	3,10	-0.0452	0.33	1.3700
	Julian Date	$\log\text{CORT}_B$	0.7084	1,12	-0.0229	0.41	0.0128
		$\log\text{CORT}_{30}$	0.4058	1,12	-0.0479	0.54	0.0084

		CORT <sub>30</sub> - CORT <sub>B</sub>	0.215	1,12	-0.0643	0.65	0.4604	
	Decimal Time	logCORT <sub>B</sub>	2.306	2,10	0.2316	0.93	0.0099	
		logCORT <sub>30</sub>	2.383	3,10	0.2419	0.11	0.1576	
		CORT <sub>30</sub> - CORT <sub>B</sub>	0.822	3,10	-0.0452	0.49	5.7150	
Roof Characteristics	Parapet Height (cm) <sup>S</sup>	logCORT <sub>B</sub>	0.676	3,9	-0.0881	0.24	0.0243	
		logCORT <sub>30</sub>	0.1931	3,9	-0.2527	0.87	-0.0029	
		CORT <sub>30</sub> - CORT <sub>B</sub>	0.8641	3,9	- 0.03516	0.91	-0.1452	
	Roof Height (m) <sup>S</sup>	logCORT <sub>B</sub>	0.676	3,9	-0.0881	0.50	0.0684	
		logCORT <sub>30</sub>	0.1931	3,9	-0.2527	0.63	0.0447	
		CORT <sub>30</sub> - CORT <sub>B</sub>	0.8641	3,9	- 0.03516	0.28	7.0126	
	Mean Gravel Diameter (cm) <sup>S</sup>	logCORT <sub>B</sub>	0.676	3,9	-0.0881	0.52	0.0303	
		logCORT <sub>30</sub>	0.1931	3,9	-0.2527	0.63	0.0207	
		CORT <sub>30</sub> - CORT <sub>B</sub>	0.8641	3,9	- 0.03516	0.27	3.3660	
	Chick Condition	Mass (g)	logCORT <sub>B</sub>	3.005	2,11	0.2358	0.74	0.0045
			logCORT <sub>30</sub>	1.411	2,11	0.0595	0.46	-0.0096
			CORT <sub>30</sub> - CORT <sub>B</sub>	0.6981	2,11	-0.0487	0.52	-0.6719
Wing Length (cm)		logCORT <sub>B</sub>	3.005	2,11	0.2358	0.06	0.0263	
		logCORT <sub>30</sub>	1.411	2,11	0.0595	0.48	0.0085	
		CORT <sub>30</sub> - CORT <sub>B</sub>	0.6981	2,11	-0.0487	0.72	0.3411	
Microclimate	Anemometer T <sub>a</sub> (°C) <sup>S</sup>	logCORT <sub>B</sub>	1.924	1,11	0.0715	0.19	0.0460	
		logCORT <sub>30</sub>	4.795	1,11	0.2403	0.05	0.0561	
		CORT <sub>30</sub> - CORT <sub>B</sub>	1.736	1,11	0.0578	0.21	2.9330	
	Anemometer Max Wind (KPH) <sup>S</sup>	logCORT <sub>B</sub>	0.665	1,11	-0.0287	0.43	0.0024	
		logCORT <sub>30</sub>	0.002	1,11	-0.0907	0.97	0.0001	
		CORT <sub>30</sub> - CORT <sub>B</sub>	0.345	1,11	-0.0578	0.57	-0.1151	
	Ovoid T <sub>e</sub> (°C) <sup>S</sup>	logCORT <sub>B</sub>	0.440	1,10	-0.0536	0.52	-0.0304	
		logCORT <sub>30</sub>	0.280	1,10	-0.0700	0.61	0.02122	
		CORT <sub>30</sub> - CORT <sub>B</sub>	0.634	1,10	-0.0344	0.44	2.3950	

Regional Climate	iButton T <sub>a</sub> (°C) <sup>S</sup>	logCORT <sub>B</sub>	21.14	1,3	0.8343	0.02	-0.4072
		logCORT <sub>30</sub>	72.91	1,3	0.9473	<0.001**	-0.3965
		CORT <sub>30</sub> -CORT <sub>B</sub>	1019	1,3	0.9963	<0.001**	-19.6065
	Maximum T <sub>a</sub> (°C) for Day	logCORT <sub>B</sub>	1.494	9,4	0.255	0.97	0.1253
		logCORT <sub>30</sub>	0.4751	9,4	-0.571	0.84	0.8098
		CORT <sub>30</sub> -CORT <sub>B</sub>	0.226	9,4	-1.154	0.96	16.748
	Maximum T <sub>a</sub> (°C) for Week	logCORT <sub>B</sub>	1.494	9,4	0.255	0.36	-0.6005
		logCORT <sub>30</sub>	0.4751	9,4	-0.571	0.93	0.6057
		CORT <sub>30</sub> -CORT <sub>B</sub>	0.226	9,4	-1.154	0.92	-6.501
	Maximum T <sub>a</sub> (°C) for 30 Days	logCORT <sub>B</sub>	1.494	9,4	0.255	0.96	0.6252
		logCORT <sub>30</sub>	0.4751	9,4	-0.571	0.88	2.6953
		CORT <sub>30</sub> -CORT <sub>B</sub>	0.226	9,4	-1.154	0.98	43.862
	Maximum Dew Point (°C) for Day	logCORT <sub>B</sub>	1.494	9,4	0.255	0.85	-0.3713
		logCORT <sub>30</sub>	0.4751	9,4	-0.571	0.79	-0.6277
		CORT <sub>30</sub> -CORT <sub>B</sub>	0.226	9,4	-1.154	0.97	-8.412
	Maximum Dew Point (°C) for Week	logCORT <sub>B</sub>	1.494	9,4	0.255	0.92	0.6496
		logCORT <sub>30</sub>	0.4751	9,4	-0.571	0.84	-1.5895
		CORT <sub>30</sub> -CORT <sub>B</sub>	0.226	9,4	-1.154	0.96	-35.883
	Maximum Dew Point (°C) for 30 Days	logCORT <sub>B</sub>	1.494	9,4	0.255	0.97	-0.4798
		logCORT <sub>30</sub>	0.4751	9,4	-0.571	0.88	-2.5868
		CORT <sub>30</sub> -CORT <sub>B</sub>	0.226	9,4	-1.154	0.98	-47.155
	Maximum Humidity (%) for Day	logCORT <sub>B</sub>	1.494	9,4	0.255	0.93	0.1393
		logCORT <sub>30</sub>	0.4751	9,4	-0.571	0.89	-0.2723
		CORT <sub>30</sub> -CORT <sub>B</sub>	0.226	9,4	-1.154	0.97	-7.264
	Maximum Humidity (%) for Week	logCORT <sub>B</sub>	1.494	9,4	0.255	0.49	-0.5454
		logCORT <sub>30</sub>	0.4751	9,4	-0.571	0.89	-0.1361
		CORT <sub>30</sub> -CORT <sub>B</sub>	0.226	9,4	-1.154	0.92	8.463
	Maximum Humidity (%)	logCORT <sub>B</sub>	1.494	9,4	0.255	0.95	0.5056
		logCORT <sub>30</sub>	0.4751	9,4	-0.571	0.85	1.9548

%) for 30 Days	CORT <sub>30</sub> -	0.226	9,4	-1.154	0.96	44.491
	CORT <sub>B</sub>					
Maximum Wind (KPH) for Day	logCORT <sub>B</sub>	0.002	1,12	-0.083	0.97	0.0009
	logCORT <sub>30</sub>	0.058	1,12	-0.078	0.81	0.0046
	CORT <sub>30</sub> - CORT <sub>B</sub>	0.2371	1,12	-0.062	0.64	0.6890
Maximum Wind (KPH) for Week	logCORT <sub>B</sub>	0.1919	1,12	-0.066	0.67	0.0384
	logCORT <sub>30</sub>	0.0018	1,12	-0.083	0.97	-0.0032
	CORT <sub>30</sub> - CORT <sub>B</sub>	0.0038	1,12	-0.083	0.95	-0.3476
Maximum Wind (KPH) for 30 Days	logCORT <sub>B</sub>	0.0447	1,12	-0.079	0.83	0.0236
	logCORT <sub>30</sub>	0.7897	1,12	-0.016	0.39	-0.082
	CORT <sub>30</sub> - CORT <sub>B</sub>	0.8513	1,12	-0.012	0.37	-6.3840

**TABLE 6:** Number of samples collected and number of samples sequenced from each location

<b>Town</b>	<b># Individuals</b>	<b># Successfully Extracted and Sequenced</b>
Elk Point	10	7
North Sioux City	17	7
Vermillion	30	17
Yankton	9	7

**TABLE 7:** Number of samples collected and number of samples sequenced by year

<b>Year</b>	<b># Individuals</b>	<b># Successfully Extracted and Sequenced</b>
2014	7	7
2015	16	8
2016	43	23

**TABLE 8:** Number of samples collected and number of samples sequenced by sample type

<b>Sample Type</b>	<b># Individuals</b>	<b># Successfully Extracted and Sequenced</b>
Adult	2	1
Chick	12	10
Egg	31	21
Blood	14	5
Feather	3	1
Fecal	4	0



**TABLE 9:** Number of samples collected and number of samples successfully extracted and sequenced by location, sample type, and collection year

Location	Type	# samples collected by year			# samples sequenced by year		
		2014	2015	2016	2014	2015	2016
Elk Point	Adult	0	0	0	0	0	0
	Chick	0	0	1	0	0	1
	Egg	0	0	6	0	0	5
	Blood	0	0	2	0	0	1
	Feather	0	0	0	0	0	0
	Fecal	0	0	1	0	0	0
North Sioux City	Adult	0	0	0	0	0	0
	Chick	0	0	1	0	0	1
	Egg	3	7	2	3	1	0
	Blood	0	0	1	0	0	1
	Feather	0	0	1	0	0	1*
	Fecal	0	0	2	0	0	0
Vermillion	Adult	0	1	0	0	0	0
	Chick	1	3	5	1	3	4
	Egg	3	3	2	3	2	2
	Blood	0	0	10	0	0	2
	Feather	0	0	1	0	0	0
	Fecal	0	0	1	0	0	0
Yankton	Adult	0	0	1	0	0	1
	Chick	0	0	1	0	0	0
	Egg	0	2	3	0	2	3
	Blood	0	0	1	0	0	1
	Feather	0	0	1	0	0	0
	Fecal	0	0	0	0	0	0

\*This sample was not used in analysis

**TABLE 10:** Gene Flow and Genetic Differentiation by subpopulation

Population	Sequences	Segregating sites (S) Synonymous, Non-synonymous	Haplotypes (h)	Haplotype Diversity (Hd)	Avg. nucleotide differences (K)	Nucleotide diversity ( $\pi$ )
<b>Elk Point</b>	7	8, 3	4	0.81	5.03	0.0230
<b>North Sioux City</b>	6	7, 0	3	0.60	2.33	0.0140
<b>Vermillion</b>	17	12, 0	6	0.82	3.41	0.0205
<b>Yankton</b>	7	7, 0	4	0.81	3.14	0.0189
<b>Total Data</b>	37	14, 3	11	0.87	3.80	0.0210

**TABLE 11:** Gene flow estimate pairwise  $F_{ST}$  values below diagonal and pairwise  $G_{ST}$  values above diagonal

	<b>Elk Point</b>	<b>North Sioux City</b>	<b>Vermillion</b>	<b>Yankton</b>
<b>Elk Point</b>	-	0.14	0.09	0.11
<b>North Sioux City</b>	0.04	-	0.08	0.05
<b>Vermillion</b>	0.15	0.03	-	0.02
<b>Yankton</b>	0.19	0.11	0.08	-